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A Distinct Acne Microbiome: Fact or Fiction?

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TO THE EDITOR

We are pleased to see that Fitz-Gibbon *et al.* (2013) set out to conduct a prospective comparison of the microbiome of acne-prone and healthy skin using elegant molecular methods. They report that the skin of acne patients is enriched by particular strains, more specifically 16S ribotypes, with a very low prevalence in healthy subjects. The authors tell us that their study 'demonstrates a previously unreported paradigm of commensal strain populations that could explain the pathogenesis of human diseases', a powerful claim that requires robust evidence to support it.

The role of *Propionibacterium acnes* in the disease after which it was named has never been conclusively proven. It is not hard to see why, given that it is a ubiquitous resident of human skin from adrenarche to old age (Leyden *et al.*, 1975) and persists long after the acne-prone years. It is the dominant bacterial resident of healthy pilosebaceous follicles (Puhvel *et al.*, 1975). Recently, it has become possible to differentiate between different strains of *P. acnes* using techniques such as pulsed-field gel electrophoresis and multilocus sequence typing (Oprica *et al.*, 2004; Lomholt and Kilian, 2010; Kilian *et al.*, 2012; McDowell *et al.*, 2012). Although no typing study has been specifically designed to look for differences between strains from acne-prone and healthy skin, some data have been interpreted to suggest that differences may exist (Lomholt and Kilian 2010; McDowell *et al.*, 2012). Fitz-Gibbon *et al.* (2013) sought to specifically address this important question.

Detailed appraisal raises doubts about the authors' methods and the conclusions drawn from them. The sample population is stated to comprise 101 subjects, 49 acne patients and 52 healthy controls, all from South California. The reader is referred to the dbGaP website (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000263.v1.p1) for further details, which show that the study sample initially comprised 148 subjects: 72 cases and 76 controls. No attempt has been made to match the age ranges of the patients and controls, and thus the oldest patient was 38 years old and the oldest control subject was 80 years old; eight control subjects were aged 42 years and above. Seven control subjects had been treated for acne in the past; it is unclear whether these had been excluded from the cohort in the published article. Antibiotic therapy is known to perturb the microflora, and hence knowledge of previous treatment is pertinent; in 18 patients, treatment histories, even for the last 12 months, were not available. Among the cases, all had facial acne, but 48 (67%) did not have acne on the nose. In acne, the nose is often spared, as is the case here. Sampling from this largely unaffected site is therefore not advisable or likely to yield the evidence required to answer the question posed. Scant details are given of how the samples were collected and processed, but enough to show that they were not ideal for comparing acne versus control subjects. Pore strips were used to obtain pooled follicular casts from the nose. In both acne and non-acne, predominantly normal follicles will have been sampled by this procedure, as they always vastly outnumber lesional ones. It is doubtful whether this method will adequately sample inflamed lesions even in the minority of patients in whom they may have been present.

The text refers to the pore tape samples as microcomedones and goes on to say that individual microcomedones were isolated using forceps. Acne is characterized by comedones of several types, of which whiteheads (closed comedones), blackheads (open comedones), and macrocomedones are the most common types (Cunliffe *et al.*, 2000). Microcomedones

Abbreviation: RT, ribotype

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in situ are not visible to the naked eye and can only be seen in electron micrographs of skin biopsies. Pore stripping, like cyanoacrylate glue, removes vellus hairs to which follicular casts are attached. These casts comprise cellular debris, sebum, and bacteria, and are typically larger when derived from acne-prone follicles. Casts from acne patients may or may not include microcomedones depending on the site sampled; those from healthy skin are certainly not microcomedones. On the nose, trichostasis spinulosa (accumulation of shed vellus hairs in the follicular lumen, Harford *et al.*, 1996) is frequently present in acne and non-acne; affected follicles will be sampled by pore stripping. It is not apparent whether the skin surface was sterilized before use of the pore tape and, as the entire tape was processed (except in the metagenomics), transient surface and resident follicular bacteria may have been included.

The results of this study show that six ribotypes were more abundant in acne patients than in controls, four significantly so. Of these six ribotypes, three (RT4, RT5, and RT10) carry a G1058C nucleotide substitution associated with resistance to tetracyclines (Ross *et al.*, 1998; Oprica *et al.*, 2005). The presence of these tetracycline-resistant variants on acne-prone skin is almost certainly a reflection of selective pressure, and not virulence. Oral tetracyclines have been a popular acne therapy for over 50 years and are still widely prescribed. Chronic antibiotic administration may or may not have created them, but it put them at a competitive advantage over susceptible lineages. Outside the United States of America, most isolates of *P. acnes* from acne patients remain tetracycline susceptible, especially in countries where tetracyclines for acne are less intensively used (Ross *et al.*, 2003; Oprica *et al.*, 2004; Luk *et al.*, 2013). In the United States of America, *P. acnes* resistant to anti-acne antibiotics were sought but not found on the skin of 1,000 acne patients sampled in the mid 1970s (Leyden, 1976); however, they were detected in the following decade (Leyden *et al.*, 1983).

The possibility that strains of *P. acnes* specifically associated with acne exist is an exciting one. Demonstrating that this is true would be a first step toward determining whether some strains are more pathogenic than others or are better able to survive within acne lesions. The authors' sophisticated molecular analyses are well suited to providing the answers as long as more appropriate samples are used.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Response to the Commentaries on the Paper: *Propionibacterium acnes* Strain Populations in the Human Skin Microbiome Associated with Acne

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TO THE EDITOR

In our recent paper, we reported our metagenomic study of the skin microbiome associated with acne (Fitz-Gibbon *et al.*, 2013). Using 16S ribosomal DNA (rDNA) analysis and whole-genome sequence analysis of 71 *Propionibacterium acnes* strains, we demonstrated that the strain populations of *P. acnes* in pilosebaceous units were distinct between acne

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